From molecular to macroscopic *via* the rational design of a self-assembled 3D DNA crystal

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A formidable hurdle to understanding the structure and function of both engineered and naturally occurring proteins that would not ordinarily crystallize, as well as other nano-particles, is that these individual tiny objects produce very weak signals when probed with techniques such as X-rays and electron beams. A straightforward way to amplify the measurable data is to arrange many such molecules or particles in a periodic array. Unfortunately, because no generalized scaffold exists for positioning nano-objects into 2D or 3D arrays, scientists are forced to rely on a tedious search for rare conditions which cause each object to self-assemble into a predictable bonding network. We sought a general technique that could orient many different types of objects into a periodic array.

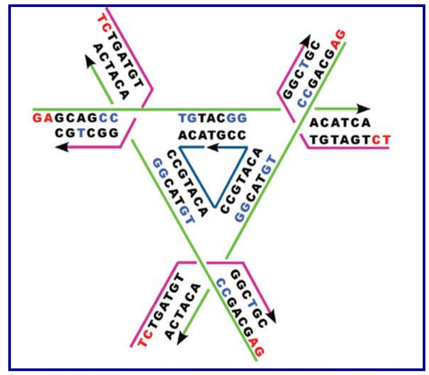

Illustration 1: Sequence and schematic design of the trefoil, showing the designed double helices and the sticky ends. The color coding for the Rapi-Data logo shows the three individual chains in the structure.

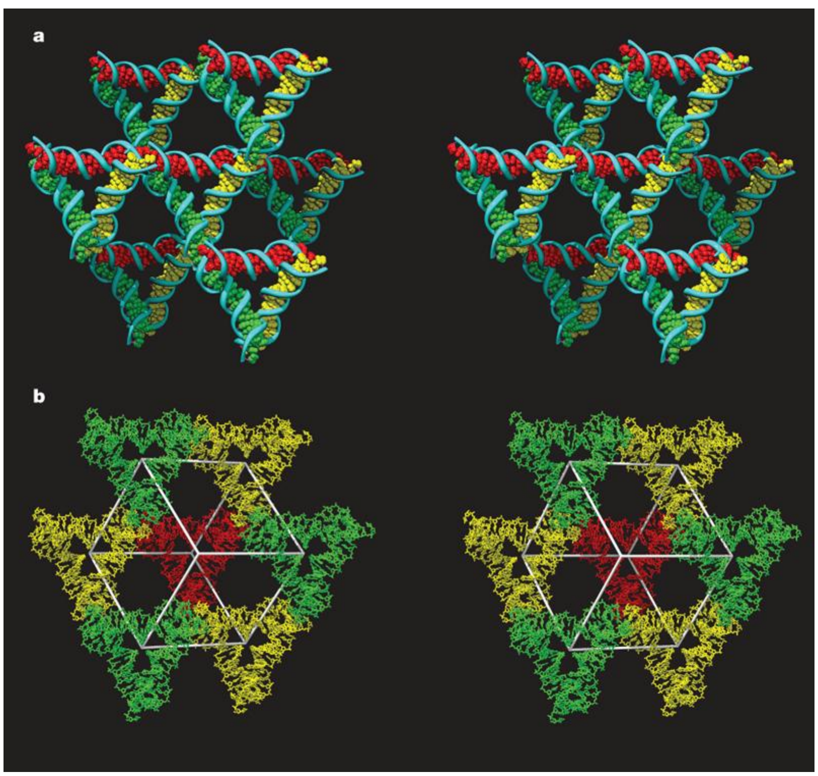

Illustration 2: Stereoscopic view of the packing within the crystal of the trefoil or tensegrity triangle. Here the red, yellow, and green chains in **a** *represent apparently continuous helices that really are individual segments within a single triangle, each of which is linked by sticky ends.*

DNA is one promising building block for assembling 2D or 3D scaffolds. By using the natural tendency of double-stranded DNA to self-assemble predictably in a shape that matches each building block with its correct partner, driven by base paring, one can design DNA to self assemble into any desired shape. We can also easily attach sticky ends to each building block so that the pieces spontaneously assemble into a rigid 3D scaffold.

Using X-ray crystallography, we confirmed the predicted and highly stable, three dimensional, completely artificial DNA structure shown in these figures. We imagine filling interstices of this "host" structure with small "guest" molecules as a way to immobilize them for crystallographic studies. We have used this design to generate crystals with even larger unit cells, and corresponding larger intra-lattice cavities. This would permit incorporation of even larger guest molecules, such as proteins and DNA assemblies. Such studies are currently in progress.

This particular project has been underway for more that 12 years. In the early stages, different design motifs, where the number of nucleotides, the type of cross-overs, and the length of sticky ends, were varied. Crystals were occasionally obtained, but they diffracted only poorly, if at all. Frequently, unit cell and symmetry parameters indicated that the predicted lattice was not obtained. These early experiments, mainly the effort of Pam Constantinou (RapiData 2001), through numerous redesigns eventually resulted in crystals that diffracted to perhaps 10-12 Å. (NSLS X8C, X12C & X29) Importantly, they had the proper rhombohedral symmetry and unit cell dimensions.

Subsequently Jianping Zheng (RapiData 2007) took over the project, and via further redesign of the initial motif was able to grow native and iodinated derivative crystals. Diffraction data were collected at X6A and X25; the phase solution was solved via SAD; and after refinement the structure shown in the logo and in Figure 2 above was obtained. Tong Wang (RapiData 2006) has been involved in work on similar triangular designs whose asymmetric units are twice as big as this specific one. These structures have all been solved and show the same motif as obtained with the triangle shown above.

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These co-authors of this publication are graduates of RapiData: Pamela Constaninou (2001), Tong Wang (2006), and Jianping Zheng (2007). **See their personal statements on the next three pages!**

I modified the initial motif, grew native and derivative crystals, collected and processed the data, and found the phase solution.

I attended the Rapidata 2007 course. It was really a great learning experience for me to learn about practical protein crystallography. We actually sat side by side with the greatest experts of crystallography in the world using the latest crystallography software with the data collected during the course. Software, such as HKL2000, Phenix, Coot, and hkl2map helped us a lot to find the structure solution easier when we got diffraction data at the end of 2007. The fundamental crystallography tutorials and crystal mounting training were very helpful too. Communication and discussion with students and professors with different research focus was a great chance to enrich knowledge in crystallography.

I joined the 3D structural project as a fresh Ph.D. student and worked on DNA molecules different from this triangle design. I also worked on similar triangular designs whose asymmetric units are twice as big as this specific one. Larger asymmetric units will create bigger space in crystals, which can host more and bigger molecular guests in crystalline lattices for structural purposes.

I‘m appreciative of having the opportunity of participating in the Rapidata course. It provides both solid background knowledge and hands-on experience to me. The course systematically introduced crystallographic software packages for different purposes: data collection, reduction, initial model building and refinement, etc. It provided the opportunity to use those packages and communicate with professionals, including the authors of the software packages. Rapidata also provides a friendly environment in which we got to know more people in structural biology, share what we know, and learn what we don’t know.

I believe I participated in Rapidata in 2001. I remember that I drove to BNL with the crystals still in the hanging drops while avoiding all bumps along the L.I.E.! At Rapidata (even though our crystals did not diffract!), I learned about mounting and storing frozen crystals. There were workshops specifically about types of cryoprotectants to try, and techniques to use for mounting. That got us in a different mode on our future trips to BNL. We were no longer wasting time trying to mount crystals during precious beamtime- we arrived with many frozen crystals ready to go.